Phytochemical and Pharmacological Studies of *Nyctanthes arbor-tristis*

SHORT SYNOPSIS

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By

Prerna Sharma

Registration number: 18/PhD/089

Under the supervision of

Dr. Abhilasha Shourie

Professor, Department of Biotechnology

MANAV RACHNA
Vidyanatarikshat
MANAV RACHNA INTERNATIONAL UNIVERSITY

Faculty of Engineering & Technology

MANAV RACHNA INTERNATIONAL INSTITUTE OF RESEARCH & STUDIES

SECTOR-43, SURAJKUND –DELHI ROAD, FARIDABAD – 121001

FACULTY OF ENGINEERING & TECHNOLOGY

Certificate

I hereby declare that the short synopsis "**Phytochemical and Pharmacological Studies of *Nyctanthes arbor-tristis***" being submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Biotechnology under the Faculty of Engineering & Technology of Manav Rachna International Institute of Research & Studies, Faridabad during the academic year 2020-21.

It is a bona fide record of original work carried out under the supervision of Dr. Abhilasha Shourie, Professor, Department of Biotechnology, FET, MRIIRS, and has not been submitted in full or part to any other university or institute for degree or diploma. Further, it is certified that short synopsis is plagiarism free and proof has been submitted.

(Prerna Sharma)
Registration number- 18/PhD/089

Supervisor
(Dr. Abhilasha Shourie)
Professor, Department of Biotechnology,
FET, MRIIRS

Joint-Supervisor **(NA)**

(Chairperson, DRC)

Chairperson, CDP

ABSTRACT

Plants extend a multitude of services to human kind and plant derived medicines have been found efficient in fighting against numerous existing and emerging diseases. It has been proven time and again that plant derived medicines have an edge above synthetic medicine with detrimental side effects. *Nyctanthes arbor-tristis* is a plant of high ethno-medicinal importance and it has been extensively used in manytraditional medicine systems for treating a wide spectrum of diseases varying from skin ailments, gynaecological disorders, ulcers, parasitic diseases, cough and fever. Although the existing studies reasonably highlight the pharmacological potential of *Nyctanthes arbor-tristis*, a more extensive research is required to explore its untapped pharmaceutical potential. This study aims at investigating *Nyctanthes arbor-tristis*for its detailed phytochemical profiling unraveling the phytochemical composition of its parts, and conducting an in-depth study involving wide spectrum analysis of pharmacological activities of its phytochemicals.

Keywords: *Nyctanthes arbor-tristis*, phytochemical profiling, anti-oxidant potential, enzyme inhibition, pharmacological activity.

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1. Introduction

Humanity has fought numerous battles with diseases, some of them happened to be the most deadly ones that wiped out huge proportion of human population all across the globe. Plants have not only catered to most needs of human beings but have been the mainstay of our existence. The phytochemicals present in the plants have been utilized by the human beings since time immemorial. Ancient civilizations all across the globe have discovered and utilized the plant medicines in ways coherent with their culture, tradition and climatic conditions. Ethno-medicinal systems developed in various parts of the world denote ancient wisdom about the use of plants in treating various disorders. Indigenous populations have depended on the forest resources for the maladies affecting them. The relationship between the plants and humans has been an intricate one that gave a chance to humans and plants to co-evolve. The co-evolution of humans and plants not only facilitated the physical adaptations in humans but also the biochemical adaptations. Plants have a variety of mechanisms which can lead to biosynthesis of many phytochemicals with significant medicinal properties such as compounds from Shikimic Acid Pathway (Schaal, 2019). Tamiflu an antiviral drug used to fight influenza is produced from Shikimic acid obtained from *Illicium verum*or Chinese Star Anise (Lenora et al., 2016). There are many more examples where bioactive compounds obtained from plant extracts are used as phytomedicines or precursors to various medicines. Taxol is an anticancer drug obtained from various *Taxus* species (Zhou et al., 2019).

1.1 Pharmacological uses of medicinal plants

Biological resources have been exploited for their medicinal, agricultural and other uses since ages. The developments in science and technology have led to a more organized process of utilizing the biological resources and tapping the hitherto untapped potential of the biological resources. Biotechnology paves way to production of novel drugs from the biological resources (Jefferey, 2002). Therefore, exploration of biological resources especially the medicinal plants opens the flood gates of possibilities of designing drugs for existing and newly emerging diseases. Medicinal plants have been an integral part of indigenous ethno-medicinal systems

and further exploration of medicinal plants can reveal novel phytochemicals or bioactive molecules present in plants, leading to new discoveries in pharmaceutics and medicine.

1.2 Phytochemicals as Pharmaceutical and therapeutic agents

Plants are repositories of various chemical compounds broadly categorized as primary and phytochemicals. The primary metabolites like carbohydrates, lipids, amino acids, proteins and nucleotides are directly involved in the growth and development processes of the plant such as nutrient assimilation, respiration and photosynthesis. Phytochemicals not only support many physiological functions but also play vital role in protection of plants against various biotic and abiotic stresses. Phytochemicals are thus instrumental in enabling the plant have a defense system against the plant eating animals, pests and pathogens protecting them from wounding or infection (Bennet and Wallsgrove, 1994).

Phytochemicals consist of a wide array of thousands of chemical compounds that exhibit huge diversity. They can be a great source of phytochemicals required by the growing pharmaceutical industry in wake of the consistent need of the humanity for innovative drugs to overcome the health challenges. Identification of new phytochemicals can lead to production of new innovative drugs that can offer solution to a health crisis. Sometimes the unique combination of phytochemicals present in a specific plant family, genus and species, attach characteristic taxonomic features that help in classification of plants (Thrane, 2001). Major classes of phytochemicals present in plants are terpenoids, alkaloids and phenolics. These phytochemicals have various pharmacologically important activities viz. anti-viral, anti- bacterial, anti-cancer, anti-diabetic etc. This is where metabolite profiling of plants is required to explore the potential of innumerous plant metabolites as drugs, involving their thorough extraction, isolation and meticulous analysis (Kopka et al., 2004).

1.3 *Nyctanthes arbor-tristis*

Ethno-botanical description and distribution

Nyctanthes arbor-tristis is commonly grown in the Indian subcontinent and is known for its beautiful fragrant flowers that blossoms in night and fall before the day break. It belongs to

family Oleaceae and grows upto the height of 10 m as a small tree or shrub. Its leaves are simple and opposite, flowers are fragrant having white, 8-lobed corolla, usually clustered, fruit is a capsule.

The plant is widely distributed in tropical and subtropical regions all across the globe. In India *Nyctanthes arbor-tristis* is mainly found in Himalayas including Jammu & Kashmir, Nepal to eastern part of Assam, West Bengal and Tripura stretching all through the central region going to southern part of India till Godavari. *Nyctanthes arbor-tristis* is commonly known as Harsingar, Night jasmine, Parijaat in Sanskrit. It is considered to be sacred plant believed to be brought on earth from the heaven. The plant has been mentioned in various ancient scriptures suggesting its medicinal value apart from the spiritual value associated with it. There are sacred groves in India and other parts of the world those have religious and cultural beliefs associated with them and have been protected by the indigenous populations. This was a way to conserve the biodiversity that was facilitated through mythological stories about the sacred groves attaching spiritual significance to them. In a study conducted in Namakkal district, Tamil Nadu, India for the sacred groves, it was found that *Nyctanthes arbor-tristis* is enumerated as a plant amongst the sacred groves and is known by the vernacular name "Pavalla Malli" (Chinnappan, 2019).

Ethno-medicinal uses

Nyctanthes arbor-tristis has been utilized in preparing various medicinal decoctions in ancient times against a wide array of diseases and thus finds its mention in Ayurveda. Owing to high therapeutic potential of the plant, it has been integrated into many traditional medicine systems other than Ayurveda like Siddha and Unani, where the phytochemicals derived from *Nyctanthes arbor-tristis* were used as an effective expectorant, laxative and diuretic. Substantial documentation reveals that all the plant parts of *Nyctanthes arbor-tristis* have been found to have therapeutic value associated with them thus establishing the plant as the one with significant pharmacological activities. Traditionally the plant was used as an effective anti- bilious agent possessing significant hepato-protective activity besides being used as a remedy for gynecological disorders. Ethno-medicinal use of *Nyctanthes arbor-tristis* in central part of India included extensive use of various plant parts in curing sores, cough, dysentry and

snakebite. Research studies have further established the anticancer, anti-inflammatory, antidepressant, antiviral, analgesic, antipyretic activities in plant extracts of *Nyctanthes arbor-tristis.*

Parasitic diseases have threatened the human population since a long time and the indigenous people had come up with plant based solution to it. The extract prepared from the shoot tips and leaves of *Nyctanthes arbor-trisis* was used to cure malaria in the Similipal Biosphere reserve of Orissa, India (Thatoi et al., 2008). According to a study conducted by Shubhangi and Patil (2011) the stem bark powder was used as an effective medicine for the treatment of asthma by the ethnic population of Jalgaon district, Maharashtra, India. Amongst the ancient sciences of medicine *Nyctanthes arbor-tristis* has been used as a part of Unani medicine to treat various disorders (Girach et al., 1994). Paste prepared by the leaves of *Nyctanthes arbor-tristis* was used by the Garasia tribe of Rajasthan, India to treat skin ailments such as ring worm and *Nyctanthes arbor-tristis* in combination with other plants was used by them to treat fever, goitre, laryngitis and stomach ache (Meena and Yadav, 2011). Juice of *Nyctanthes arbor-tristis* leaves mixed with the juice of ginger, holy basil leaves and honey was found to be effective against cough in traditional medicine in Bangladesh (Tumpa et al., 2014). Root paste of *Nyctanthes arbor-tristis* was used to treat fractures (Upadhya et al., 2012). The paste prepared with the leaves of *Nyctanthes arbor-tristis* was applied on forehead to treat fever by the indigenous population of Tharu tribe in Uttar Pradesh, India (Kumar et al., 2006). Besides using the leaf juice of *Nyctanthes arbor-tristis* to treat fever, the people of Sonapur in Assam used its seed paste to treat baldness and flowers to treat helminthes, the parasitic worms (Sharma and Sharma, 2010). In a study conducted on ethno-medicinal plants of importance from Assam region in India it was mentioned that the root extract of *Nyctanthes arbor-tristis* was used in treating inconsistency in menstruation and other gynecological problems. In yet another study on ethno-medicinal plants from Orchha region in Madhya Pradesh, *Nyctanthes arbor-tristis* leaves and flowers were used to cure cancer, prolonged heavy menstrual bleeding, ulcers, fever and sores (Jitin et al., 2013).

There is enough evidence originating from the various ethno-medicinal studies on the therapeutic potential of *Nyctanthes arbor-tristis*. This plant emerges as the one that can be a promising choice for bioprospecting it for its pharmacological activities and further use in production of novel drugs for the modern day maladies. *Nyctanthes arbor-tristis* is a rich repository of pharmacologically important phytochemicals, thus its secondary metabolite profiling leading to identification and isolation of its pharmacologically important bioactive compounds can open new vistas in drug discovery.

2. Literature Review

The ethno-medicinal studies emphasize the therapeutic importance of *Nyctanthes arbor-tristis* and all its parts have been found to have remarkable pharmacological activity that renders this plant to be extending remedy to a wide spectrum of diseases varying from skin ailments, gynecological disorders, ulcers, parasitic diseases, cough and fever. The immense pharmacological significance of *Nyctanthes arbor-tristis* has been a subject of great interest in the scientific community as the phytochemicals obtained from *Nyctanthes arbor-tristis* can be used to obtain potential drugs against several diseases.

2.1 Anti-cancer Activity

A study conducted by Timsina and Nadumane (2016) revealed the efficacy of *Nyctanthes arbor-tristis* flower and leaves extract in fighting the human cervical cancer by having strong inhibitory effect on cancer cells and no detrimental effect on the normal cells. An MTT assay performed by Grover et al. (2015) led to establishing the anti-cancer potential of *Nyctanthes arbor-tristis* leaves thus providing a scientific basis aligned with the traditional use of the plant parts as an anticancer drug. High anti-angiogenic activity was found by Pawar et al. (2016) in the calyx of *Nyctanthes arbor-tristis* flowers. Anti-angiogenic activity can lead to effectively managing and treating diseases like cancer, retinopathy resulting due to diabetes and cataract. In a study conducted by Manawadu et al. (2018) flower extract obtained from *Nycatnthes arbor-tristis* exhibited anti-proliferative activity when tested on human cancer cell and emerged as a potential drug for chronic lymphocytic leukemia.

2.2 Anti-oxidant and Anti-inflammatory Activity

In a study involving efficacy of flower extracts of *Nyctanthes arbor-tristis* in various solvents, it was found that the ethanolic extract exhibited maximum antioxidant activity. The rich presence of antioxidants in flower extracts of *Nyctanthes arbor-tristis* could be a way to provide natural antioxidants at a low cost (Mishra et al., 2016). Yet another study by Ghosh et al. (2015) emphasizes the significant antioxidant activity demonstrated by F2 polysaccharide obtained from the leaves of *Nyctanthes arbor-tristis*. High anti-oxidant and hepatoprotective activities were proven by Chaudhary et al. (2018) in the leaf extract *Nyctanthes arbor-tristis*. Noteworthy anti-oxidant and anti-proliferative activity has been reported by Khanapur et al. (2014) in the flower extract of *Nyctanthes arbor-tristis*, the investigation also led to finding of two important phytochemicals Beta-monogentiobioside and Arborside C in the flower extracts. Significantly high phenolic content of *Nyctanthes arbor-tristis* leaves was found to possess anti free radical activity (Ladumor et al., 2017). A multitude of assays conducted by Dhinakaran and Sakhtivel (2016) confirmed anti-oxidant and anti-inflammatory activity of various plant parts of *Nyctanthes arbor-tristis*. An investigation led by Patel and Gokhale (2016) reemphasized the immense anti-oxidant activity possessed by the *Nyctanthes arbor-tristis* plant parts.

A study conducted by Lad and Bhatnagar (2017) emphasized the anti-oxidant potential of the leaf extract of *Nyctanthes arbor-tristis* owing to the presence of flavonoid, triterpenoid and phenolic compounds. The extract strengthened the antioxidant defense and attenuated the effects of arthritis considerably. Noteworthy anti-oxidant capacity was established by Sundaram et al. (2018) in the leaf extract of *Nyctanthes arbor-tristis* due to the presence of phytochemical PHF1, which was found to be a potential anticancer agent besides also being effective in preventing skin ageing and restoring the skin elasticity. Significant activity against cardiotoxicity has been recorded by Mahajan and Mohan (2016) in plant extracts of *Nyctanthes arbor-tristis* when tested on wistar rats. This activity against cardiotoxicity is attributed to the noteworthy antioxidant potential of Nyctanthes arbor-tristis.

Traditionally *Nyctanthes arbor-tristis* was widely used to treat rheumatism and inflammatory diseases, specifically arthritis. In an investigation performed by Uroos et al. (2017) it was found

that ethyl acetate extracts of *Nyctanthes arbor-tristis* leaves were significantly efficient in inhibiting rheumatoid arthritis.

2.3 Anti -depressant Activity

Depression is a common disorder affecting a large population all across the globe and is prevalent in almost all the age groups. Mild depression might lead to serious mental illness sometimes. Studies indicate that indigenous people have been treating and managing depression and other mental disorders with the extracts obtained from medicinal plants. *Nyctanthes arbor-tristis* has been indicated to have anti-depressant activity that was tested on mice and construes it to be a potential anti-depressant drug (Gupta et al., 2016).

2.4 Bioactivity against Neurodegenerative disorders

There is steep rise in neurodegenerative disorders amongst the elderly population all across the globe. Flower extracts of *Nyctanthes arbor-tristis* prepared in ethanol have found to be possessing Acetylcholine-esterase activity that leads to reduction of oxidative stress in the brain tissue thus exhibiting ameliorative activity (Mishra et al., 2018).

2.5 Anti-helminthic Activity

Nyctanthes arbor-tristis leaf extract is found to be significantly efficacious against Indian earthworm thus proving its anti-helminthic properties that could be exploited in manufacturing drugs for helminthic diseases (Ahmed et al., 2016).

2.6 Immuno-modulatory Activity

Both ex vivo and in vivo test conducted on mice revealed the effective immunomodulatory activity of *Nyctanthes arbor-tristis* flower extract making the plant a suitable contender for potential immunostimulant drugs that enhance the immunity (Bharshiv et al., 2016). In times when there are diseases like Covid-19 for which no vaccination or drug has yet been manufactured, use of *Nyctanthes arbor-tristis* flower extracts or a combination of plant immune-stimulants could be a great option to boost immunity thus protecting the human

population from various diseases including Covid-19. In a study conducted by Alamgir and Uddin (2010) the leaf extract of *Nyctanthes arbor-tristis* has been found to show notable immune-modulatory activity by restoring the suppressed immunological aspects in rats exposed to a chemical malathion. Significant immune-modulatory activity has been reported in *Nyctanthes arbor-tristis* leaf extract by Kannan et al. (2007), the study was conducted on rats and notable rise in the antibody concentration was found when the rats were administered the leaf extract of *Nyctanthes arbor-tristis*.

2.7 Anti-diabetic Activity

Diabetes is a growing threat that needs to be treated with plant based medicines offering natural therapy with practically no side effects. The leaf extract of *Nyctanthes arbor-tristis* have been found to exhibit hypoglycemic and hypolipidemic activities, which could be utilized in treating diabetes (Rangika et al., 2015).

2.8 Analgesic activity

Analgesic activity has been reported in *Nyctanthes arbor-tristis* leaves by Pattanayak and Datta (2013). In an investigation led by Kakoti et al. (2013) *Nyctanthes arbor-tristis* bark extract was found to possess effective anti-inflammatory and analgesic properties when tested on rats.

2.9 Anti-microbial Activity

Remarkable antimicrobial activity has been detected in the bark extract of *Nyctanthes arbor-tristis* by Paarakh et al. (2016) when tested against a wide variety of microorganisms. Bark extracts have exhibited significant antifungal activities and anti-bacterial activity, antibacterial activity was also prominently found in leaves extract of *Nyctanthes arbor-tristis* owing to the presence of phytochemical Beta-sitosterol in leaves. Noteworthy activity against salmonella was found in *Nyctanthes arbor-tristis* (Pasha et al., 2009)

2.10 Hepato-protective Activity

Hepatitis a major cause of liver cirrhosis leads to high number of deaths all over the world. *Nyctanthes arbor-tristis* has been used to treat liver diseases according ancient medicinal system. A study by Tripathi et al. (2015) corroborates the hepato-protective activity of *Nycthanthes arbor-tristis* stem bark when tested on rats.

2.11 Pharmacological Activity due to Endophytic Fungi

Endophytic fungi are considered to be of great importance as they are sources of numerous phytochemicals and bioactive compounds those can revolutionize the discovery of drugs from biological products. An endophytic fungus i.e., *Aspergillus nidulans* has been found to be living in association with *Nyctanthes arbor-tristis*. The metabolites obtained from *A. nidulans* were found to be having significant anti-proliferative activity when tested with human cancer lines (Sana et al., 2019). A study by Mishra et al. (2015) establishes the cytotoxic potential of a secondary metabolite, altersolanol A extracted from an endophytic fungus *Phomopsis sp* found in *Nyctanthes arbor-tristis*. Altersolanol A has been found to be considerably effective against human cancer cell lines.

2.12 Role in modern medicine

Nanoparticles are revolutionizing the drug delivery systems and diagnostics (Zhang et al., 2008). There have been attempts to create nanomedicine from the extracts of medicinal plants that would be target oriented and more effective. In one such development the *Nyctanthes arbor-tristis* seed extract has been used to prepare silver nanoparticles in an ecofriendly way (Basu et al., 2016).

2.13 Insilico Studies on *Nyctanthes arbor-tristis*

In molecular docking study conducted by Heendeniya et al. (2020) compounds 4- hydroxypyridine 1-oxide and phenol,2,5-bis (1,1- dimethylethyl) exhibited significant specificity towards a protein called survivin thus demonstrating a possibility of *Nyctanthes arbor-tristis* as

a potential drug against leukemia. This paves way to establishing the effectiveness of *Nyctanthes arbor-tristis* flower extract in inhibiting the proliferation of human leukemia cells.

3. Description of Broad Area

The findings of the literature review offer ample evidence that the various plant parts of *Nyctanthes arbor-tristis* have been utilized by the ethnic communities worldwide as a remedy for several diseases. Based on the ethno-medicinal history of the plant, there have been consistent attempts made by the scientific community to validate the therapeutic activities associated with various plant parts of *Nyctanthes arbor-tristis*. The literature survey reveals that a number of researches have been conducted for identification and isolation of phytochemicals and analysis of the pharmacological activities of *Nyctanthes arbor-tristis* such as antimicrobial, anti-proliferative, anticancer, anti-diabetic and many other. However, such researches often are restricted to exploration of limited bioactivity profile of its crude extracts or partially fractionated extracts. Though there have been fair amount of work done in order to exhibit the biological activities of *Nyctanthes arbor-tristis* still there is a huge gap that needs to be covered so that the phytochemicals obtained from *Nyctanthes arbor-tristis* can be established as potential drugs playing a pivotal role in curing the diseases. In view of the exceptional ethno- pharmacological importance of *Nyctanthes arbor-tristis*, this study has been planned to carry out in-depth studies involving a detailed phytochemical profiling of its parts and analysis of pharmacological activities of potential compounds.

4. Objectives

The objectives of this study are-

1. To purify and characterize phytochemicals from *Nyctanthes arbor-tristis*.
2. To predict the bioactivity of some purified phytochemicals of *Nyctanthes arbor-tristis* through in-silico approach.
3. To assess the in-vitro pharmacological activity of *Nyctanthes arbor-tristis* phytochemicals.

5. Methodology

5.1 Procurement of Plant Material

The plants of *Nyctanthes arbor-tristis* will be purchased from Medicinal plant garden at The Energy and Resources Institute(TERI), Gurgaon and the voucher specimen will be deposited at NBPGR, New Delhi following proper identification and authentication.

5.2 Sample preparation

Different plant parts (stem, leaves, roots, flowers and seeds) will be shade dried separately at room temperature and will be finely powdered. The powdered material will be stored in dark and dry conditions until further use.

5.3 Extraction of Phytochemicals

Crude extracts will be prepared using solvents of different polarities through appropriate extraction method such as Soxhlet extraction, ultra-sonication or microwave assisted extraction. The crude extracts will then be partitioned using different solvents on the basis of polarity of compounds. The fractions obtained from different solvents will be separately concentrated in a rotary evaporator and will be stored in a refrigerator until further use.

5.4 Phytochemical Screening

Each of the fractions will be tested for the presence of various classes of phytochemicals viz. tannins, saponins, flavonoids, terpenoids, alkaloids, cardiac glycosides and anthraquinones, following standard biochemical testing methods described by Harborne (1998).

5.5 Quantitative Phytochemical analysis

Total Polyphenols

As demonstrated by Meduoa et al. (2009) total polyphenols in the extracts will be determined by spectrophotometric method using the Folin-Ciocolteau reagent and the quantity will be expressed as Gallic Acid Equivalents using a gallic acid standard curve.

Total flavonoid content

Total flavonoid content in the extracts will be determined by aluminium chloride method and the absorbance values will be measured. Flavonoid content will be expressed as Catechin Equivalents using a catechin standard curve (Lola-Luz et al., 2014)

Tannin content

Each sample will be mixed in acidified acetone- methanol solution and will be sonicated and centrifuged at 6000 rpm for 10 min. The supernatant will be incubated with acidified butanol to give red colour. The absorbance will be recorded at 550 nm (Tlili et al., 2019).

Carotenoid content

Powdered plant material will be mixed with the solvent mixture chloroform: ethanol: diethyl ether solution and will be saponified using potassium hydroxide solution. It will be extracted again with chloroform: ethanol solution and assayed spectrophotometrically at 450 nm, using a standard (Kurilich and Juvik, 1999).

Saponin content

As demonstrated by Kaur et al. (2015) total saponin content will be determined using gravimetric method involving partitioning of methanolic plant extracts in aqueous n-butanol solution. Saponin extract will be concentrated by evaporation of the solvent and will be dried in an oven to obtain a constant weight. The saponin content will be calculated as:

$$\% \text{ Saponin} = \frac{\text{Weight of saponin}}{\text{Weight of sample}} \times 100.$$

Alkaloid content

According to Biradar and Rachetti (2013) to determine the total content of alkaloids the sample will be added to acetic acid and ethanol solution, stirred for 2 days, filtered, and concentrated at 2.0 pH. This solution will be subjected to separation and precipitation to evaluate the percentage of alkaloids present in the sample.

$$\% \text{ Alkaloid} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100.$$

5.6 Purification of Phytochemicals

Column Fractionation of Crude extracts

The extracts obtained using different solvents will be chromatographed in a column packed with Silica gel (60-120 mesh) using appropriate solvent combinations in a step gradient manner. About 10-20 equi-volume fractions (10 ml each) will be collected for each gradient solvent. All the fractions will be chromatographed using thin layer chromatography (TLC) and the fractions containing similar compounds on the basis of TLC will be pooled together.

Identification and characterization of Phytochemicals-

The bioactive fractions will be characterized and individual bioactive compounds will be identified by using techniques- HPLC, LC–ESI-MS/MS, GC-MS, FTIR, H^1 NMR and C^{13} NMR.

5.7 Antioxidant Potential

DPPH Radical Scavenging

To determine the DPPH radical scavenging power, measured volumes of different extracts will be added to 1 ml of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. Samples will be incubated and the absorbance will be measured at 517 nm against a methanol control and the results will be expressed as EC_{50} (effective concentration, mg/ml) derived by plotting the concentration of the tested sample with the percentage of radical scavenging activity (Moon and Shibamoto, 2009).

FRAP test

The reducing power of the plant extracts will be determined using the FRAP test. Each plant extract will be added to a reaction solution with phosphate buffer and potassium ferricyanide. After incubation, centrifugation and addition of trichloroacetic acid, the supernatant was mixed

distilled water and $FeCl_3$. The absorbance will be measured at 700 nm and EC_{50} value (mg/ml) will be calculated (Benzie and Strain, 1996).

Cupric ion reducing antioxidant capacity (CUPRAC assay)

The solutions of plant extracts will be added to reaction mixture of $CuCl_2$, neocuproine, ammonium acetate buffer for the absorbance to be registered at 450nm after incubation. Similar procedure is followed for a blank sample. The results will be expressed in Trolox equivalents (Apak et al., 2006).

Phosphomolybdenum assay

Extract solution will be added to reaction mixture consisting of sulphuric acid, sodium phosphate and ammonium molybdate and incubated at 95 °C for one hour. The absorbance will be recorded against a blank at 695 nm and antioxidant capacity was expressed as Trolox equivalents (Prieto et al., 1999).

Metal ion chelating activity

To determine the metal ion (ferrous) chelating potential of the plant extracts, samples will be added to a reaction mixture consisting of $FeSO_4$ solution, Tris–HCl buffer, bipyridyl solution, hydroxylamine hydrochloride and ethanol. EDTA or Citric acid will be used as standard. After incubation, absorbance will be measured at 522nm and percentage metal chelation will be calculated as follows (Adinortey et al., 2018)-

Metal ion chelating activity = $[(A_{control} - A_{sample})/ A_{sample}] \times 100$

Hydroxyl radical scavenging activity (HRSA)

To assess the hydroxyl radical scavenging activity the reaction mixture will consist of different concentrations of plant extracts, iron-EDTA solution, DMSO and ascorbic acid. After subjecting to water bath treatment and termination of reaction by TCA, Nash reagent will be added to the reaction mixture that will be incubated to develop yellow colour. The intensity of yellow colour

will be recorded against a blank reagent at 412nm and the percentage HRSA was calculated as follows (Adinortey et al., 2018)-

% HRSA = $[(A_{control} - A_{sample})/ A_{sample}]$ X 100

Nitric oxide radical scavenging activity

To determine the nitric oxide (NO) radical scavenging activity the extracts will be added to the sodium nitroprusside solution prepared in phosphate buffer. Then the Griess reagent (1% sulfanilamide and 0.1% naphthylenediamine dichloride in 5% phosphoric acid) will be added to the mixture and it will be further incubated. The absorbance will be measured at 540 nm against blank and the percentage inhibition of NO will be calculated as follows (Adinortey et al., 2018)-

% NO radical scavenging activity = $[(A_{control} - A_{sample})/ A_{sample}]$ X 100

Lipid peroxidation inhibition assay

Lipid peroxidation inhibition will be measured by thiobarbituric acid reactive species (TBARS) assay. The extracts will be added to dilute egg yolk homogenate and will be incubated with Fe_2SO_4.Then 20% acetic acid, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) will be added and the mixture will be heated in a water bath shaker. The TBA-MDA complex will be extracted in butanol and its absorbance will be measured at 532 nm and lipid peroxidation inhibition activity will be calculated as follows (Adinortey et al., 2018)-

% Lipid peroxidation inhibition activity = $[(A_{control} - A_{sample})/ A_{sample}]$ X 100

Superoxide anion scavenging assay

According to Sharma and Singh (2012) the superoxide anion scavenging potential of extracts will be assessed by measuring the reduction of nitro blue tetrazolium (NBT). The reaction mixture will consist of NBT, NADH and phenazine methosulphate (PMS) in sodium phosphate buffer along with the plant extract. The reaction mixture will be incubated at room temperature for 10 min and absorbance will be recorded at 560 nm against blank as a measure

of NBT reduction. Ascorbic acid will be used as standard. The percentage of superoxide radical scavenging will be calculated as follows-

Superoxide radical scavenging activity (%) = $[(A_{control}-A_{sample})/A_{control} \times 100]$

5.8 Enzyme inhibitory activity

Proteinase inhibitory action

Some modification in a method reported by (Oyedapo and Famurewa, 1995) is used to perform proteinase inhibitory action the reaction mixture containing trypsin, Tris-HCl buffer and plant extracts will be subjected to incubation with casein. The reaction will be terminated by adding perchloric acid and absorbance will be read at 210 nm against blank. Percentage inhibition of proteinase will be calculated as follows-

Percentage proteinase inhibition = $[(A_{control}-A_{sample})/A_{control} \times 100]$

α-Amylase inhibition

The α-Amylase inhibitory activity will be conducted using the Caraway–Somogyi iodine/potassium iodide (IKI) method. Absorbance will be measured at 630 nm against blank and enzyme activity will be calculated (Lazarova et al., 2015).

α-Glucosidase inhibition

The α-Glucosidase inhibitory activity will be conducted by incubating the reaction mixture consisting of extract solutions, glutathione, α-glucosidase solution in phosphate buffer and p-Nitrophenyl-α-glucopyranoside (PNPG) as substrate. The reaction will be stopped by sodium carbonate and absorbance will be recorded at 400nm and enzyme activity will be calculated (Lazarova et al., 2015).

Tyrosinase inhibition

Tyrosinase inhibitory activity will be conducted using the modified dopachrome method. The extracts will be added to the tyrosinase solution and phosphate buffer in a 96-well microplate and L-DOPA will be added as a substrate. The reaction mixture will be then incubated for 15

min at 25 °C. The absorbance will be recorded at 492 nm against blank and enzyme activity will be calculated (Zengin et al., 2014)

Acetylcholinesterase inhibition

According to Khadri et al. (2010) to perform acetylcholinesterase inhibition activity the extracts will be mixed with Tris-HCl Buffer and acetylcholinesterase solution and incubated in 96 well plates. Further acetylcholine iodide and DTNB will be added to the reaction mixture. The absorbance will be measured at 405 nm and inhibition percentage will be calculated as follows-

$$\mathrm{I}\,(\%) = 100 - (\mathrm{A\ sample}/\mathrm{A\ control}) \times 100$$

5.9 Molecular docking studies

Molecular docking studies will be done for selected phytochemicals of *Nyctanthes arbor-tristis* to evaluate their binding interactions with pharmacologically implicated macromolecular receptors.

6. Expected Outcome

This study will enable us to identify and characterize the phytochemicals present in *Nyctanthes arbor-tristis.* Solvent fractionation of the crude plant extracts will yield certain purified bioactive compound which will be tested for their biological activities such as anti-oxidant, enzyme inhibitory, and other pharmacological activities. A detailed phytochemical and pharmacological profiling of *Nyctanthes arbor-tristis* will lay a foundation for discovery of novel drugs which might prove useful in treatment of various diseases.

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